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# CHEMICAL & PHARMACEUTICAL BULLETIN



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# 2-Arylmethyl-1,4-benzoquinones. I. Novel Inhibitors of Platelet Aggregation: Synthesis and Pharmacological Evaluation[1)]

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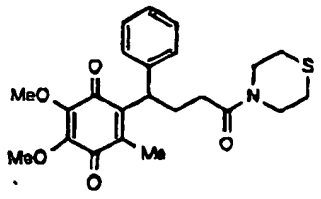
**A new series of 2-arylmethyl-1,4-benzoquinones (2) was synthesized for evaluation of their pharmacological activities. These compounds showed significant inhibition of platelet aggregation induced by arachidonic acid (AA) and some of them possessed a protective effect against endothelial cell injury caused by hydrogen peroxide.**

Key words 1,4-benzoquinone; anti-platelet aggregation; arachidonic acid; thromboxane; hydrogen peroxide; endothelial cell

As part of our research on cerebral protective agents, we have already reported the synthesis and pharmacological evaluation of 4-(1,4-benzoquinon-2-yl)-4-arylbutanamide (1) (SUN4757) and some analogues.[1)] We were interested in the effect of introducing an alkylcarboxylic acid or alkylamide group onto the benzene ring on the biological properties of these compounds. This led us to synthesize various 2-arylmethyl-1,4-benzoquinones (2) [$R_1$=Me or MeO, $R_2$, $R_3$=H, $(CH_2)_nCOOR$ or $(CH_2)_nCONR_2$, $n$=2, 3] and to evaluate their pharmacological profile. All of them showed a significant decrease in cerebral protective activity compared to the parent compound (1), so we examined other biological activities of these novel compounds (2). They were found to be inhibitors of platelet aggregation induced by arachidonic acid (AA). It is well known that the AA metabolite thromboxane $A_2$ ($TXA_2$) is a potent inducer of platelet aggregation and of vascular and pulmonary smooth muscle contraction.[2)] It has also been implicated as an important mediator in a variety of diseases, such as myocardial infarction, inflammation, asthma and stroke. During our study, (±)-7-(3,5,6-trimethyl-1,4-benzoquinon-2-yl)-7-phenylheptanoic acid (3) (AA-2414), which possesses similar functional groups in the molecule, was reported as a novel type of eicosanoid receptor antagonist[3,4)] and it was suggested that both a benzoquinonyl moiety and a carboxylic acid moiety, with appropriate distance, were essential for exhibiting a significant activity. We therefore became interested in investigating the structural requirements for anti-platelet aggregation activity of the compounds (2) (Fig. 1). Here we report the synthesis and pharmacological evaluation of new 2-phenylmethyl-1,4-benzoquinone derivatives which exhibit marked anti-platelet aggregation activity.

**Chemistry** The synthetic procedures for the target compounds are summarized in Charts 1—3. The 3,5,6-trimethyl-2-phenylmethyl-1,4-benzoquinone derivative (2a) was prepared from the benzaldehyde derivative (4). Reaction of 4 with the Grignard reagent prepared from 2-(3-bromophenyl)-1,3-dioxolane, followed by acetylation

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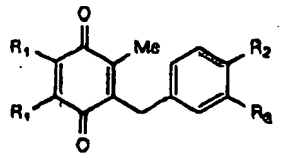
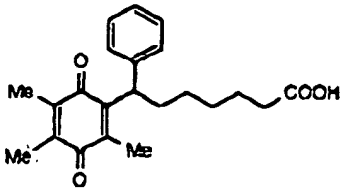
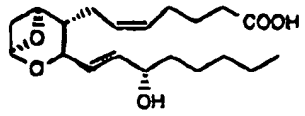



2




3: (AA-2414)




$TXA_2$

Fig. 1

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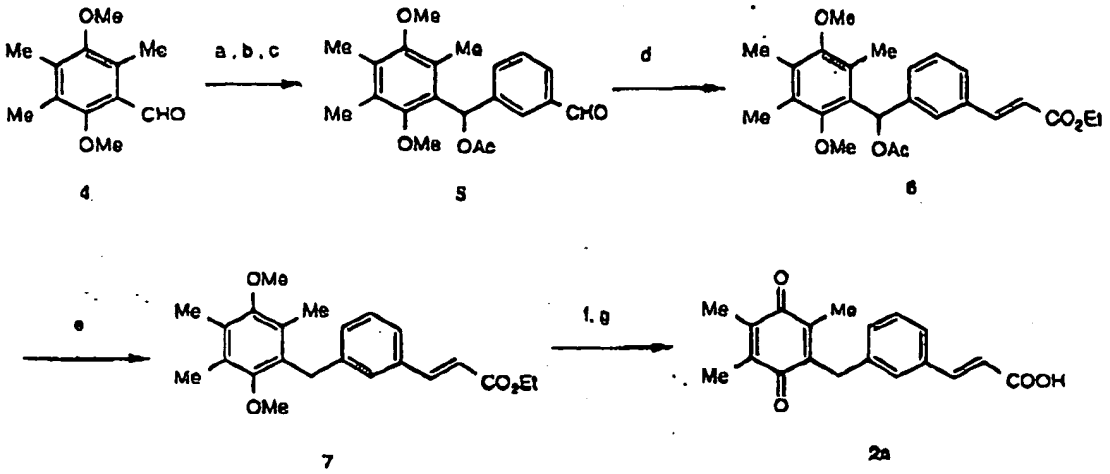
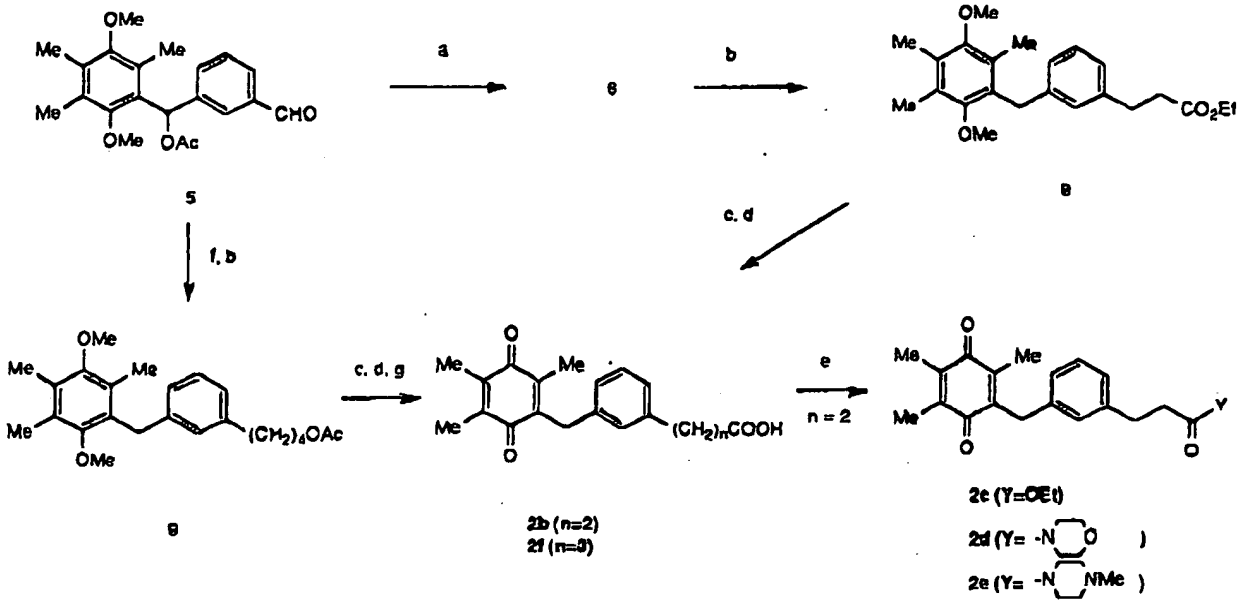



a: 2-(3-bromophenyl)-1,3-dioxolane, Mg b: $Ac_2O$, pyridine, DMAP c: *p*-TsOH, acetone d: $(EtO)_2POCH_2CO_2Et$, NaH e: $Et_3SiH$, TMSOTf f: 2N NaOH g: CAN

Chart 1



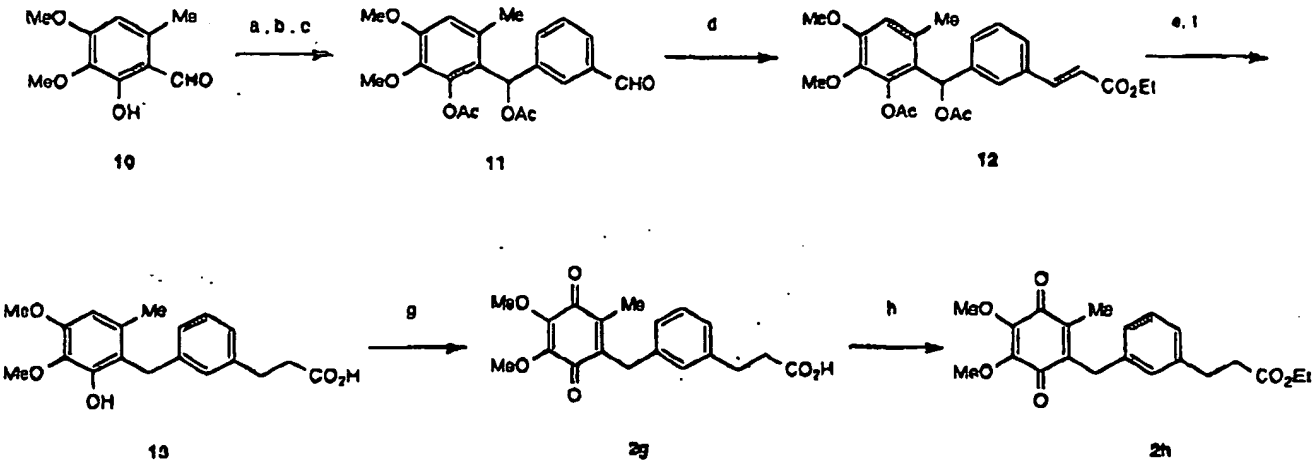

a: $(EtO)_2POCH_2CO_2Et$, NaH b: $H_2$, Pd, AcOH c: 2N NaOH d: CAN e: HOR or $HNR_2$, DMAP, DCC f: $[Ph_3P(CH_2)_3OH]Cl$, NaH g: $CrO_3$, $H_2SO_4$

Chart 2

and acetal exchange reaction afforded the aldehyde derivative (**5**). A coupling reaction of **5** and triethyl phosphonoacetate yielded the $\alpha,\beta$-unsaturated carboxylate (**6**) (*E*-isomer), which was converted to **7** by reduction with triethylsilane in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) as a catalyst. Hydrolysis of **7**, followed by oxidation with cerium ammonium nitrate (CAN)[5] afforded the benzoquinone derivative (**2a**) (Chart 1).

Hydrogenolysis of **6** in acetic acid using palladium–black as a catalyst afforded **8**, which was transformed to the benzoquinone derivative (**2b**) ($n=2$) by a similar procedure to that described above. Compound **2b** was transformed to the ester (**2c**) or amides (**2d**, **2e**) by condensation with the appropriate alcohol or amines. Wittig reaction of **5** and triphenyl-3-hydroxypropylphosphonium chloride, followed by hydrogenolysis in acetic acid afforded the acetate derivative (**9**). Hydrolysis of **9**, followed by oxidation with CAN and Jones' reagent afforded **2f** ($n=3$) (Chart 2).

The 5,6-dimethoxy-3-methyl-2-phenylmethyl-1,4-benzoquinone derivative (**2g**) was prepared from salicylaldehyde (**10**). Compound **10** was converted to **11** by a similar method to that used for the synthesis of **5**. Coupling reaction of **11** and triethyl phosphonoacetate, followed by hydrogenolysis and hydrolysis afforded **13**. The obtained



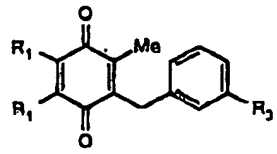

10 11 12

13 2g 2h

a: 2-(3-bromophenyl)-1,3-dioxolane, Mg b: $Ac_2O$, pyridine, DMAP c: *p*-TsOH, acetone d: $(EtO)_2POCH_2CO_2Et$, NaH e: $H_2$, Pd, AcOH f: KOH g: salcomine, $O_2$ h: HOR, DMAP, DCC

Chart 3

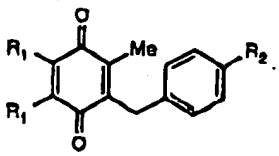
Table 1. Physical Data for 2a—h ($R_2$=H)




| Compound | $R_1$ | $R_3$ | Yield (%) | mp (°C) | Formula | Analysis (%) Calcd C | Calcd H | Calcd N | Found C | Found H | Found N |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2a | Me | CH=CHCOOH | 85 | 146—148 | $C_{19}H_{18}O_4$ | 73.53 | 5.85 | | 73.45 | 5.80 | |
| 2b | Me | $(CH_2)_2COOH$ | 72 | 110—112 | $C_{19}H_{20}O_4$ | 73.06 | 6.45 | | 72.64 | 6.48 | |
| 2c | Me | $(CH_2)_2COOEt$ | 68 | a) | $C_{21}H_{24}O_4$ | 74.09 | 7.11 | | 74.01 | 7.13 | |
| 2d | Me | $(CH_2)_2$-C(=O)-N⟨O⟩ | 62 | 75—77 | $C_{23}H_{27}NO_4$ | | 381.1940 b) | | | 381.1909 b) | |
| 2e | Me | $CH_2)_2$-C(=O)-N⟨NMe⟩ | 55 | 141—143 | $C_{24}H_{30}N_2O_3$ | 73.07 | 7.66 | 7.10 | 73.00 | 7.74 | 7.05 |
| 2f | Me | $(CH_2)_3COOH$ | 76 | a) | $C_{20}H_{22}O_4$ | | 326.1518 b) | | | 356.1528 b) | |
| 2g | MeO | $(CH_2)_2COOH$ | 69 | 119—121 | $C_{19}H_{20}O_6$ | 66.27 | 5.85 | | 66.30 | 5.84 | |
| 2h | MeO | $(CH_2)_3COOEt$ | 75 | a) | $C_{21}H_{24}O_6$ | 67.73 | 6.50 | | 67.85 | 6.64 | |

a) Obtained as an oil. b) Determined by high-resolution mass spectrometry.

Table 2. Physical Data for 2i—m ($R_3$=H)




| Compound | $R_1$ | $R_2$ | Yield (%) | mp (°) | Formula | Analysis (%) Calcd C | Calcd H | Calcd N | Found C | Found H | Found N |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2i | Me | CH=CHCOOH | 63 | 225—228 | $C_{19}H_{18}O_4$ | 73.53 | 5.85 | | 73.29 | 5.78 | |
| 2j | Me | CH=CHCOOEt | 89 | a) | $C_{21}H_{22}O_4$ | 74.54 | 6.55 | | 74.58 | 6.60 | |
| 2k | Me | $(CH_2)_2COOH$ | 70 | 154—156 | $C_{19}H_{20}O_4$ | 73.06 | 6.45 | | 72.55 | 6.38 | |
| 2l | Me | $(CH_2)_3COOH$ | 69 | a) | $C_{20}H_{22}O_4$ | | 326.1518 b) | | | 326.1546 b) | |
| 2m | MeO | $(CH_2)_2COOH$ | 62 | a) | $C_{19}H_{20}O_6$ | 66.27 | 5.85 | | 66.32 | 5.90 | |

a) Obtained as an oil. b) Determined by high-resolution mass spectrometry.

phenol derivative (13) was transformed to the benzoquinone derivative (2g) by oxidation with Fremy's salt (or catalytic air oxidation).[6] Compound 2g was converted to the ester (2h) by condensation with alcohol (Chart 3).

Regioisomers of the above-mentioned compounds were also synthesized similarly. Chemical structures of the synthesized compounds were determined on the basis of spectroscopic data [infrared (IR), proton nuclear magnetic resonance ($^1$H-NMR), and mass (MS) spectra] and elemental analyses. The physical data are summarized in Tables 1 and 2.

**Pharmacological Evaluation** Anti-platelet aggregation activities of the benzoquinone derivatives described above were measured in terms of their ability to inhibit platelet aggregation induced by collagen, AA and adenosine diphosphate (ADP), as described by Born.[7] The results are displayed in Table 3 as $IC_{50}$ values (the concentation needed to inhibit platelet aggregation by 50%).

The $\alpha,\beta$-unsaturated carboxylic acid (2a) ($R_2$=H, $R_3$=CH=CHCOOH) was inactive against collagen-, AA- and ADP-induced platelet aggregation even at a high concentration ($IC_{50}$=>200 $\mu$g/ml). Compound 2i ($R_2$=CH=CHCOOH, $R_3$=H), a regioisomer of 2a, showed no anti-platelet aggregation activity. Although the $\alpha,\beta$-saturated carboxylic acid (2k) [$R_2$=$(CH_2)_2$COOH, $R_3$=H] was also ineffective, 2b [$R_2$=H, $R_3$=$(CH_2)_2$COOH], a regio-isomer of 2k, was found to show a significant activity against platelet aggregation induced by AA ($IC_{50}$=3.4 $\mu$g/ml). This compound was inactive against collagen- or ADP-induced aggregation ($IC_{50}$=>200 $\mu$g/ml). To investigate the structure–activity relationships (SAR) for AA-induced platelet aggregation, modifications were made in the quinonyl moiety, the carboxylic acid moiety and the alkylene chain length in the molecule. Modification of the benzoquinone ring, such as replacement of the methyl group of 2b ($R_1$=Me) by a methoxy group (2h) ($R_1$=MeO) resulted in loss of activity ($IC_{50}$=50 $\mu$g/ml).

Modification of the carboxylic acid moiety, such as conversion to the ester (2c) ($IC_{50}$=7 $\mu$g/ml) or amide (2d) ($IC_{50}$=13 $\mu$g/ml) resulted in a reduction of activity. In addition, modification of the methylene chain length between the benzene ring and carboxylic acid moiety also resulted in a reduction of activity, as in the case of 2f [$R_2$=H, $R_3$=$(CH_2)_3$COOH] ($IC_{50}$=14 $\mu$g/ml). In contrast, similar modification of the methylene chain length of an inactive compound (2k) improved the activity, as in the case of compound (2l) [$R_2$=$(CH_2)_3$COOH, $R_3$=H] ($IC_{50}$=28 $\mu$g/ml). In summary, 3-{3-[(3,5,6-trimethyl-1,4-benzoquinon-2-yl)methyl]phenyl}propionic acid (2b) was found to be the most potent inhibitor of AA-induced platelet aggregation in this series. The results of chemical modification suggest that the existence of a flexible carboxyl group on the benzene ring of the 2-phenylmethyl-1,4-benzoquinone nucleus is important for exhibiting the anti-platelet aggregation activity.

An assay for protective activity against endothelial cell injury caused by hydrogen peroxide was also conducted since the generation of free radicals is observed in some cardiovascular diseases and it is believed that such radical species may damage the cardiac tissues and the vessels.

Table 3. Pharmacological Evaluation of 2a—m

| Compd. | Anti-platelet aggregation ($IC_{50}$; $\mu$g/ml) | | | LDH release (inhibition %) | |
|---|---|---|---|---|---|
| | Collagen | AA | ADP | 10 $\mu$M | 1 $\mu$M |
| 2a | >200 | >200 | >200 | | |
| 2b | >200 | 3.4 | >200 | 78 | 35 |
| 2c | 33 | 7.0 | >200 | | |
| 2d | 35 | 13 | 200 | 65 | 20 |
| 2e | 66 | 13 | >200 | 78 | 20 |
| 2f | 33 | 14 | 220 | | |
| 2g | >200 | 9.5 | >200 | | |
| 2h | 208 | 50 | 220 | | |
| 2i | >200 | >200 | >200 | | |
| 2j | >200 | >200 | >200 | | |
| 2k | >200 | >200 | >200 | 88 | 45 |
| 2l | 220 | 28 | >200 | 75 | 30 |
| 2m | >200 | >200 | >200 | | |

Selected compounds in this series were evaluated in this screening assay and their protective activities were evaluated as inhibition % of lactate dehydrogenase (LDH) release from endothelial cells as described by Abe *et al.*[8] Compound 2b was found to show a protective activity in this screening test (78% inhibition at 10 $\mu$M). Thus, new candidates for our project have been found. Further SAR studies and pharmacological experiments are under way and the results will be published elsewhere.

**Experimental**

Melting points were determined on a Yanaco melting point apparatus and are uncorrected. The $^1$H-NMR spectra were recorded on a JEOL JNM-GX270 spectrometer, using tetramethylsilane as an internal standard, and IR spectra were obtained with either a Hitachi 260-10 or a Nicolet 5DX instrument. Elemental analyses were performed on a Perkin-Elmer 240B elemental analyzer. MS were obtained with a Hitachi M80 instrument with a direct inlet system.

**3-{Acetoxy-[(2,5-dimethoxy-3,4,6-trimethyl)phenyl]methyl}benzaldehyde (5)** A solution of 2,5-dimethoxy-3,4,6-trimethylbenzaldehyde (3.50 g, 16.8 mmol) in tetrahydrofuran (THF) (150 ml) was added to a solution of Grignard reagent [prepared from 2-(3-bromophenyl)-1,3-dioxolane (12.2 g, 95%, 50.6 mmol) and magnesium (1.27 g, 52.3 mmol)] in THF (200 ml) under ice-cooling, followed by stirring at room temperature for 4 h. The reaction mixture was poured into water and extracted with ether. The organic solution was washed with water, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by silica-gel column chromatography with hexane–ethyl acetate (3:1) to afford 2-{3-[hydroxy-[(2,5-dimethoxy-3,4,6-trimethyl)phenyl]methyl}phenyl}-1,3-dioxolane (4.32 g, 12.07 mmol). This compound (2.50 g, 6.98 mmol) was dissolved in a mixture of acetic anhydride (855 mg, 8.38 mmol), pyridine (622 mg, 8.38 mmol), 4-dimethylaminopyridine (DMAP) (85 mg, 0.70 mmol) and methylene chloride (100 ml), and the mixture was stirred at room temperature for 16 h, then washed with 5% aqueous hydrochloric acid and water, dried and concentrated. The crude product was purified by silica-gel column chromatography with hexane–ethyl acetate (3:1) to afford the acetate derivative. Then this compound (1.02 g, 2.55 mmol) and *p*-toluenesulfonic acid monohydrate (*p*-TsOH) (40 mg) were dissolved in acetone (80 ml) and the solution was stirred at room temperature for 6 h. After concentration, the residue was diluted with ether and the solution was washed with saturated $NaHCO_3$ and water, dried and concentrated to afford 5 (853 mg, 2.40 mmol). $^1$H-NMR ($CDCl_3$) $\delta$: 2.12 (3H, s), 2.22 (6H, s), 2.24 (3H, s), 3.62 (3H, s), 3.71 (3H, s), 7.20—7.85 (5H, m), 9.98 (1H, s). IR ($CHCl_3$): 1732, 1697 cm$^{-1}$. MS *m/z*: 356 ($M^+$).

By a similar procedure, 4-{acetoxy-[(2,5-dimethoxy-3,4,6-trimethyl)-phenyl]methyl}benzaldehyde was synthesized from 2-(4-bromophenyl)-1,3-dioxolane and 2,5-dimethoxy-3,4,6-trimethylbenzaldehyde. $^1$H-NMR ($CDCl_3$) $\delta$: 2.13 (3H, s), 2.22 (3H, s), 2.24 (3H, s), 3.62 (3H, s),

3.71 (3H, s), 7.20—7.85 (5H, m), 9.98 (1H, s). IR ($CHCl_3$): 1732, 1697 $cm^{-1}$. MS *m/z*: 356 ($M^+$).

**Ethyl 3-(3-(Acetoxy-[(2,5-dimethoxy-3,4,6-trimethyl)phenyl]methyl)-phenyl)acrylate (6)** A solution of triethylphosphonoacetate (743 mg, 3.32 mmol) in THF (50 ml) was treated with sodium hydride (133 mg, 60%, 3.33 mmol), followed by stirring at room temperature for 40 min. Then a solution of 5 (853 mg, 2.40 mmol) in THF (30 ml) was added under ice-cooling and the mixture was stirred at room temperature for 16 h. The reaction mixture was poured into water and extracted with ether. The extract was washed with water, dried and concentrated. The crude product was purified by silica-gel column chromatography with hexane–ethyl acetate (3 : 1) to afford 6 (801 mg, 1.88 mmol). $^1$H-NMR ($CDCl_3$) $\delta$: 1.33 (3H, t), 2.12 (3H, s), 2.22 (9H, s), 3.63 (3H, s), 3.69 (3H, s), 4.26 (2H, q), 6.38 (1H, d), 7.10—7.50 (4H, m), 7.58 (1H, s), 7.63 (1H, d). IR ($CHCl_3$): 1726, 1701, 1638 $cm^{-1}$. MS *m/z*: 426 ($M^+$). By a similar procedure, ethyl 3-{4-{acetoxy-[(2,5-dimethoxy-3,4,6-trimethyl)-phenyl]methyl}phenyl}acrylate was synthesized. $^1$H-NMR ($CDCl_3$) $\delta$: 1.33 (3H, s), 2.12 (9H, s), 3.62 (3H, s), 3.69 (3H, s), 4.24 (2H, q), 6.40 (1H, d), 7.18 (2H, d), 7.43 (2H, d), 7.58 (1H, s), 7.64 (1H, d). IR ($CHCl_3$): 1728, 1704, 1637 $cm^{-1}$. MS *m/z*: 426 ($M^+$).

**Ethyl 3-(3-[(2,5-Dimethoxy-3,4,6-trimethylphenyl)methyl]phenyl)acrylate (7)** A solution of 6 (684 mg, 1.61 mmol) in methylene chloride (30 ml) was added to a solution of triethylsilane (224 mg, 1.93 mmol) and TMSOTf (60 mg, 0.27 mmol) in methylene chloride (50 ml) at room temperature over 30 min, followed by stirring at the same temperature for 20 min. The reaction mixture was washed with water, dried and concentrated. The crude product was purified by silica-gel column chromatography with hexane–ethyl acetate (4 : 1) to afford 7 (543 mg, 1.48 mmol). $^1$H-NMR ($CDCl_3$) $\delta$: 1.32 (3H, t), 2.09 (3H, s), 2.22 (6H, s), 3.55 (3H, s), 3.64 (3H, s), 4.07 (2H, s), 4.24 (2H, q), 6.36 (1H, d), 7.00—7.40 (4H, m), 7.61 (1H, d). IR ($CHCl_3$): 1699, 1636 $cm^{-1}$. MS *m/z*: 368 ($M^+$).

By a similar procedure, ethyl 3-{4-[(2,5-dimethoxy-3,4,6-trimethyl-phenyl)methyl]-phenyl}acrylate was synthesized. $^1$H-NMR ($CDCl_3$) $\delta$: 1.32 (3H, t), 2.09 (3H, s), 2.22 (6H, s), 3.55 (3H, s), 3.64 (3H, s), 4.07 (2H, s), 4.25 (2H, q), 6.36 (1H, d), 7.11 (2H, d), 7.39 (2H, d), 7.63 (1H, d). IR ($CHCl_3$): 1704, 1638 $cm^{-1}$. MS *m/z*: 368 ($M^+$).

**3-(3-[(3,5,6-Trimethyl-1,4-benzoquinon-2-yl)methyl]phenyl)acrylic Acid (2a)** A solution of 7 (122 mg, 0.33 mmol) in a mixture of 2 N aqueous sodium hydroxide (1.6 ml) and 1,4-dioxane (0.4 ml) was heated under reflux for 2 h. The reaction mixture was diluted with water, acidified and extracted with ether. The extract was washed with water, dried and concentrated. The product was dissolved in a mixture of acetonitrile (4 ml) and water (1.5 ml), then CAN (291 mg, 0.53 mmol) was added at room temperature, followed by stirring at the same temperature for 30 min. The reaction mixture was diluted with water and extracted with ether. The organic solution was washed with water, dried and concentrated. The crude product was purified by silica-gel column chromatography with 5% methanol–methylene chloride to afford 2a (87 mg, 0.28 mmol). $^1$H-NMR ($CDCl_3$) $\delta$: 2.03 (6H, s), 2.11 (3H, s), 3.89 (2H, s), 6.42 (1H, d), 7.15—7.50 (4H, m), 7.73 (1H, d). IR (KBr): 2936, 1693 $cm^{-1}$. MS *m/z*: 310 ($M^+$).

By a similar procedure, 3-{4-[(3,5,6-trimethyl-1,4-benzoquinon-2-yl)methyl]phenyl}acrylic acid (2b) was synthesized. $^1$H-NMR ($CDCl_3$) $\delta$: 2.02 (6H, s), 2.10 (3H, s), 3.89 (2H, s), 6.39 (1H, d), 7.21 (2H, d), 7.45 (2H, d), 7.73 (1H, d). IR (KBr): 2964, 1692, 1648, 1628 $cm^{-1}$. MS *m/z*: 310 ($M^+$).

**Ethyl 3-(3-[(2,5-Dimethoxy-3,4,6-trimethylphenyl)methyl]phenyl)propionate (8)** A solution of 6 (630 mg, 1.48 mmol) in acetic acid (5 ml) was added to a suspension of palladium–black (prepared from 200 mg of palladium chloride) in acetic acid (30 ml), followed by stirring under a hydrogen gas stream at room temperature for 16 h. After filtration, the filtrate was concentrated under reduced pressure and the residue was purified by silica-gel column chromatography with hexane–ethyl acetate (3 : 1) to afford 8 (471 mg, 1.38 mmol). $^1$H-NMR ($CDCl_3$) $\delta$: 1.22 (3H, s), 2.09 (3H, s), 2.21 (3H, s), 2.22 (3H, s), 2.55 (2H, t), 2.88 (2H, t), 3.53 (3H, s), 3.64 (3H, s), 4.03 (2H, s), 4.10 (2H, q), 6.80—7.30 (4H, m). IR ($CHCl_3$): 1730 $cm^{-1}$. MS *m/z*: 370 ($M^+$). By a similar procedure, ethyl 3-{4-[(2,5-dimethoxy-3,4,6-trimethylphenyl)methyl]phenyl}propionate was synthesized. $^1$H-NMR ($CDCl_3$) $\delta$: 1.22 (3H, t), 2.10 (3H, s), 2.21 (3H, s), 2.22 (3H, s), 2.55 (2H, t), 2.88 (2H, t), 3.53 (3H, s), 3.64 (3H, s), 4.03 (2H, s), 4.10 (2H, q), 6.80—7.30 (4H, m). IR ($CHCl_3$): 1730 $cm^{-1}$. MS *m/z*: 370 ($M^+$).

**3-(3-[(3,5,6-Trimethyl-1,4-benzoquinon-2-yl)methyl]phenyl)propionic Acid (2b)** A solution of 8 (350 mg, 0.95 mmol) in a mixture of 2 N aqueous sodium hydroxide (20 ml) and dioxane (10 ml) was heated at 70 °C for 3 h. The reaction mixture was diluted with water, acidified and extracted with ether. The extract was washed with water, dried and concentrated. The crude product was dissolved in a mixture of acetonitrile (30 ml) and water (10 ml), then CAN (1.32 g, 2.41 mmol) was added at room temperature, followed by stirring at the same temperature for 1 h. The reaction mixture was diluted with water and extracted with ether. The organic solution was washed with water, dried and concentrated under reduced pressure. The residue was purified by silica-gel column chromatography with 5% methanol–methylene chloride to afford 2b (211 mg, 0.68 mmol). $^1$H-NMR ($CDCl_3$) $\delta$: 2.02 (6H, s), 2.08 (3H, s), 2.64 (2H, t), 2.90 (2H, t), 3.84 (2H, s), 6.90—7.30 (4H, m). IR (KBr): 3000, 1708, 1642 $cm^{-1}$. MS *m/z*: 312 ($M^+$). HR-MS: Found 312.1347 (Calcd 312.1362).

By a similar procedure, 3-{4-[(3,5,6-trimethyl-1,4-benzoquinon-2-yl)methyl]phenyl}propionic acid (2k) was synthesized. $^1$H-NMR ($CDCl_3$) $\delta$: 2.01 (6H, s), 2.09 (3H, s), 2.64 (2H, t), 2.90 (2H, t), 3.83 (2H, s), 7.10 (4H, s). IR (KBr): 3000, 1710, 1642 $cm^{-1}$. MS *m/z*: 312 ($M^+$).

***N*-(3-[3-(3,5,6-Trimethyl-1,4-benzoquinon-2-yl)methyl]phenyl)propionyl Morpholine (2d)** 1-Ethyl-3-(3-dimethylamino-propyl)carbodiimide hydrochloride (28 mg, 0.15 mmol) was added to a solution of 2b (30 mg, 0.10 mmol) and morpholine (11 mg, 0.13 mmol) in methylene chloride (5 ml) at room temperature, followed by stirring at the same temperature for 5 h. The reaction mixture was washed with water, dried and concentrated. The crude product was purified by silica-gel column chromatography with hexane–ethyl acetate (1 : 1) to afford 2d (31 mg, 0.08 mmol).

**4-(3-[(2,5-Dimethoxy-3,4,6-trimethylphenyl)methyl]phenyl}butyl Acetate (9)** Compound 5 (810 mg, 2.28 mmol) was added to a solution of Wittig reagent, prepared from triphenyl-3-hydroxypropylphosphonium bromide (4.56 g, 11.37 mmol) and sodium hydride (455 mg, 60%, 11.38 mmol) in THF (50 ml), followed by stirring under reflux for 16 h. The reaction mixture was concentrated under reduced pressure and the residue was purified by silica-gel column chromatography with hexane–ethyl acetate (1 : 1) to afford 4-{3-[acetoxy-(2,5-dimethoxy-3,4,6-trimethylphenyl)methyl]phenyl}-3-buten-1-ol (560 mg, 1.41 mmol). This compound (540 mg, 1.36 mmol) was added to a suspension of palladium–black (200 mg) in acetic acid (10 ml) and the mixture was stirred at room temperature for 16 h. After filtration, the filtrate was concentrated under reduced pressure and the residue was purified by silica-gel column chromatography with hexane–ethyl acetate (5 : 1) to afford 9 (410 mg, 1.07 mmol). $^1$H-NMR ($CDCl_3$) $\delta$: 1.50—1.74 (4H, m), 2.04 (3H, s), 2.09 (3H, s), 2.21 (3H, s), 2.22 (3H, s), 2.45—2.65 (2H, m), 3.53 (3H, s), 3.63 (3H, s), 4.04 (2H, s), 3.95—4.15 (2H, m), 6.80—7.20 (4H, m). IR ($CHCl_3$): 1730 $cm^{-1}$. MS *m/z*: 384 ($M^+$).

By a similar procedure, 4-{4-[(2,5-dimethoxy-3,4,6-trimethylphenyl)-methyl]phenyl}butyl acetate was synthesized. $^1$H-NMR ($CDCl_3$) $\delta$: 1.45—1.75 (4H, m), 2.03 (3H, s), 2.10 (3H, s), 2.21 (6H, s), 2.45—2.65 (2H, m), 3.54 (3H, s), 3.64 (3H, s), 4.03 (2H, s), 3.95—4.15 (2H, m), 6.90—7.10 (4H, m). IR ($CHCl_3$): 1724 $cm^{-1}$. MS *m/z*: 384 ($M^+$).

**4-(3-[(3,5,6-Trimethyl-1,4-benzoquinon-2-yl)methyl]phenyl)butyric Acid (2f)** A solution of 9 (408 mg, 1.06 mmol) in a mixture of 2 N aqueous sodium hydroxide (5 ml) and dioxane (5 ml) was heated under reflux for 3 h. The reaction mixture was poured into water and extracted with ether. The organic solution was washed with water, dried and concentrated under reduced pressure. The crude product was dissolved in acetone (20 ml) and to this solution was added an excess of Jones' reagent under ice-cooling, followed by stirring at the same temperature for 1 h. The reaction mixture was poured into water and extracted with ether. The organic solution was concentrated and the residue was purified by silica-gel column chromatography with 5% methanol–methylene chloride to afford 4-{3-[(2,5-dimethoxy-3,4,6-trimethylphenyl)methyl]-phenyl}butyric acid (272 mg, 0.76 mmol). A solution of this compound (120 mg, 0.34 mmol) in a mixture of acetonitrile (6 ml) and water (2 ml) was treated with CAN (462 mg, 0.34 mmol) at room temperature, followed by stirring at the same temperature for 30 min. The reaction mixture was poured into water and extracted with ether. The organic solution was washed with water, dried and concentrated under reduced pressure. The crude product was purified by silica-gel column chromatography with 5% methanol–methylene chloride to afford 2f (85 mg, 0.26 mmol). $^1$H-NMR ($CDCl_3$) $\delta$: 1.80—2.10 (2H, m), 2.02 (6H, s), 2.09 (3H, s), 2.36 (2H, t), 2.63 (2H, t), 3.84 (2H, s), 6.90—7.30 (4H, m). IR ($CHCl_3$): 3000, 1710, 1643 $cm^{-1}$. MS *m/z*: 326 ($M^+$). HR-MS: Found

326.1528 (Calcd 326.1518).

By a similar procedure, 4-{4-[(3,5,6-trimethyl-1,4-benzoquinon-2-yl)methyl]phenyl}butyric acid (2l) was synthesized. $^1$H-NMR ($CDCl_3$) $\delta$: 1.80—2.10 (2H, m), 2.01 (6H, s), 2.10 (3H, s), 2.34 (2H, m), 2.61 (2H, t), 3.82 (2H, s), 6.90—7.20 (4H, m). IR (KBr): 3000, 1708, 1643 cm$^{-1}$. MS *m/z*: 326 ($M^+$). HR-MS: Found 326.1546 (Calcd 326.1518).

3-{Acetoxy-[(2-acetoxy-3,4-dimethoxy-6-methyl)phenyl]methyl}benzaldehyde (11) A solution of 2-hydroxy-3,4-dimethoxy-6-methylbenzaldehyde (2.00 g, 10.20 mmol) was added to a solution of Grignard reagent [prepared from 2-(3-bromophenyl)-1,3-dioxolane (9.35 g, 40.83 mmol) and magnesium (1.04 g, 42.80 mmol)] in THF (300 ml) under ice-cooling, followed by stirring at room temperature for 12 h. The reaction mixture was poured into water and extracted with ether. The organic solution was washed with water, dried and concentrated. The crude product was purified by silica-gel column chromatography with hexane–ethyl acetate (3:2) to afford 2-{3-[hydroxy-(3,4-dimethoxy-2-hydroxy-6-methyl)-phenyl]methylphenyl}-1,3-dioxolane (3.30 g, 9.54 mmol). This compound (2.00 g, 5.78 mmol) was dissolved in a mixture of acetic anhydride (2.10 g, 20.59 mmol), pyridine (1.83 g, 23.16 mmol), DMAP (10 mg) and methylene chloride (150 ml) and the mixture was stirred at room temperature for 6 h. The reaction mixture was washed with 5% aqueous hydrochloric acid and water, dried and concentrated. The obtained compound and *p*-TsOH (20 mg) were dissolved in acetone (50 ml) and the solution was stirred at room temperature for 24 h, then concentrated under reduced pressure, and the residue was partitioned between ether and saturated aqueous sodium bicarbonate. The organic layer was washed with water, dried and concentrated to afford 11 (1.86 g, 4.82 mmol). $^1$H-NMR ($CDCl_3$) $\delta$: 2.17 (3H, s), 2.18 (3H, s), 2.38 (3H, s), 3.79 (3H, s), 3.88 (3H, s), 6.66 (1H, s), 7.22 (1H, s), 7.35—7.55 (2H, m), 7.70—7.85 (2H, m), 10.00 (1H, s). IR (KBr): 1737, 1698, 1612 cm$^{-1}$. MS *m/z*: 386 ($M^+$).

By a similar procedure, 4-{acetoxy-[(2-acetoxy-3,4-dimethoxy-6-methyl)phenyl]methyl}benzaldehyde was synthesized. $^1$H-NMR ($CDCl_3$) $\delta$: 2.16 (3H, s), 2.18 (3H, s), 2.36 (3H, s), 3.79 (3H, s), 3.88 (3H, s), 6.66 (1H, s), 7.22 (1H, s), 7.35 (2H, d), 7.83 (2H, d), 9.99 (1H, s). IR ($CHCl_3$): 1736, 1701, 1608 cm$^{-1}$. MS *m/z*: 386 ($M^+$).

Ethyl 3-{3-[[Acetoxy-(2-acetoxy-3,4-dimethoxy-6-methyl)phenyl]methyl]phenyl}acrylate (12) A solution of triethylphosphonoacetate (755 mg, 3.37 mmol) in THF (80 ml) was treated with sodium hydride (155 mg, 60%, 3.88 mmol) at room temperature, followed by stirring at the same temperature for 40 min. Then a solution of 11 (1.00 g, 2.59 mmol) in THF (30 ml) was added under ice-cooling, followed by stirring at room temperature for 3 h. The reaction mixture was poured into water and extracted with ether. The organic solution was washed with water, dried and concentrated. The crude product was purified by silica-gel column chromatography with hexane–ethyl acetate (3:1) to afford 12 (964 mg, 2.11 mmol). $^1$H-NMR ($CDCl_3$) $\delta$: 1.34 (3H, t), 2.15 (3H, s), 2.18 (3H, s), 2.35 (3H, s), 3.80 (3H, s), 3.88 (3H, s), 4.26 (2H, q), 6.39 (1H, d, $J$=16 Hz), 6.66 (1H, s), 7.10—7.50 (5H, m), 7.64 (1H, d). IR (KBr): 1748, 1715, 1639, 1612 cm$^{-1}$. MS *m/z*: 456 ($M^+$).

By a similar procedure, ethyl 3-{4-{[acetoxy-(2-acetoxy-3,4-dimethoxy-6-methyl)-phenyl]methyl}phenyl}acrylate was synthesized.

$^1$H-NMR ($CDCl_3$) $\delta$: 1.34 (3H, t), 2.14 (3H, s), 2.19 (3H, s), 2.35 (3H, s), 3.79 (3H, s), 3.87 (3H, s), 4.26 (2H, q), 6.41 (1H, d, $J$=15.8 Hz), 6.65 (1H, s), 7.18 (1H, s), 7.19 (2H, d), 7.46 (2H, d, $J$=8.58 Hz), 7.66 (1H, d, $J$=15.8 Hz). IR ($CHCl_3$): 1770, 1712, 1634, 1608 cm$^{-1}$. MS *m/z*: 456 ($M^+$).

3-{3-[(3,4-Dimethoxy-2-hydroxy-6-methylphenyl)methyl]phenyl}propionic Acid (13) Compound 12 (140 mg, 0.31 mmol) was added to a solution of palladium–black [prepared from 100 mg of palladium chloride] in acetic acid (3 ml), followed by stirring under a hydrogen gas stream at room temperature for 16 h. After filtration, the filtrate was concentrated under reduced pressure to afford ethyl 3-{3-[(2-acetoxy-3,4-dimethoxy-6-methylphenyl)methyl]phenyl}propionate (120 mg, 0.30 mmol). This was dissolved in a mixture of 5% aqueous potassium hydroxide (3 ml) and methanol (3 ml) and the solution was stirred at room temperature for 3 h. The reaction mixture was diluted with water and washed with ether. The aqueous layer was acidified with concentrated hydrochloric acid and extracted with ether. The organic solution was washed with water, dried and concentrated to afford 13 (83 mg, 0.25 mmol).

By a similar procedure, 3-{4-[(3,4-dimethoxy-2-hydroxy-6-methylphenyl)methyl]phenyl}propionic acid was synthesized.

3-{3-[(5,6-Dimethoxy-3-methyl-1,4-benzoquinon-2-yl)methyl]phenyl}-propionic Acid (2q) A mixture of 13 (54 mg, 0.16 mmol) and salcomine (30 mg) in dimethylformamide (DMF) (3 ml) was stirred under an oxygen atmosphere at room temperature for 12 h. The reaction mixture was diluted with water and extracted with ether. The organic layer was washed with water, dried over anhydrous $MgSO_4$ and concentrated. The crude product was purified by silica-gel column chromatography with 5% MeOH–$CH_2Cl_2$ to afford 2q (39 mg, 0.11 mmol).

By a similar procedure, 3-{4-[(5,6-dimethoxy-3-methyl-1,4-benzoquinon-2-yl)methyl]phenyl}propionic acid (2m) was synthesized. $^1$H-NMR ($CDCl_3$) $\delta$: 2.09 (3H, s), 2.62 (2H, t), 2.89 (2H, t), 3.80 (2H, s), 3.99 (6H, s), 6.95—7.30 (4H, m). IR ($CHCl_3$): 3000, 1708, 1646 cm$^{-1}$. MS *m/z*: 344 ($M^+$).

**Pharmacological Evaluation** Platelet Aggregation Inhibitory Activity: Blood from male Japanese White rabbits was collected into plastic vessels containing 3.8% sodium citrate (1 volume with 9 volumes of blood). Platelet-rich plasma (PRP) and platelet-poor plasma (PPP) were prepared by centrifugation at 190 × *g* for 7 min and then at 1500 × *g* for 10 min, respectively. Platelet aggregation in PRP was measured by Born's standard turbidimetric procedure[7] using an eight-channel platelet aggregometer (PAM-8C, Mebanix, Tokyo, Japan). Activity of inhibitors (test compounds) was expressed as $IC_{50}$ ($\mu$g/ml) values, *i.e.*, doses required to inhibit the platelet aggregation response induced by collagen, arachidonic acid or ADP by 50%.

Protection against the Cell Injury Caused by Hydrogen Peroxide: A monolayer of endothelial cells (CPAE) in the stationary phase was washed with EBS (Earle's balanced salt) and incubated with EBS containing test compound plus hydrogen peroxide (100 $\mu$M) for 6 h. After the incubation, LDH released into medium was determined by a standard method.[8] Then the cells were stained with 0.02% erythrosine-B and the numbers of dead cells were counted from micrographs.

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**References**

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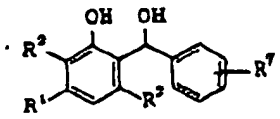
Publication Date: December 12, 2987

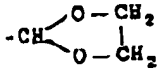
The compound represented by the general formula (I) of the present invention can be produced, for example, by the following methods:

Process I

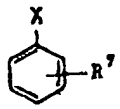
A compound represented by the general formula (IV)



(IV)

(wherein $R^1$, $R^2$ and $R^3$ each independently represents a hydrogen atom, a methyl group or a methoxy group, and $R^7$ represents a group  or $-CH(OC_2H_5)_2$ )

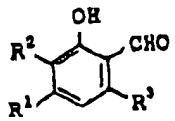
can be obtained by acting a halide Grignard reagent represented by a general formula (III)



(III)

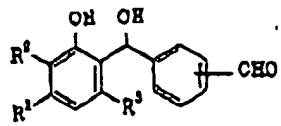
(wherein X represents a bromine atom or a chlorine atom and $R^7$ is as defined above)

to an aldehyde represented by a general formula (II)



(II)

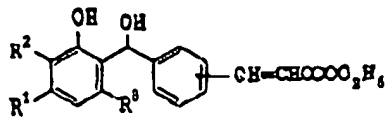
(wherein $R^1$, $R^2$, and $R^3$ are as defined above). The compound (IV) is converted into an aldehyde represented by a general formula (V)



(V)

(wherein $R^1$, $R^2$, and $R^3$ are as defined above) by treating with

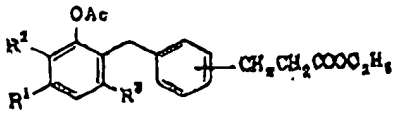
an acid, for example, hydrochloric acid. A compound represented by a general formula (VI)



(VI)

(wherein $R^1$, $R^2$, and $R^3$ are as defined above) can be obtained by acting Witting reagent of triethylphosphonoacetate to the aldehyde.

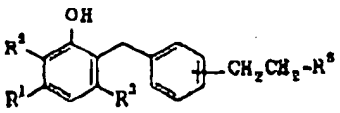
The compound (VI) is converted into an acetylated compound by reacting thereto, acetic anhydride in the presence of a base, for example, pyridine, and, subsequently, the acetylated compound is catalytically reduced in the presence of palladium black in glacial acetic acid to obtain a compound represented by a general formula (VII)



(VII)

(wherein $R^1$, $R^2$, and $R^3$ are as defined above).

The compound (VII) is subjected to hydrolysis, reduction or esterification through a conventional method to obtain a compound represented by a general formula (VIII)

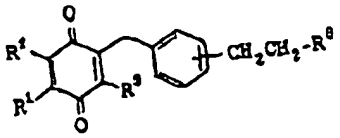


(VIII)

(wherein $R^1$, $R^2$, and $R^3$ are as defined above and $R^8$ represents a hydroxymethyl group, a carboxyl group, or a lower alkoxycarbonyl group).

Subsequently, the compound (VIII) is oxidized with oxygen in the presence of potassium nitrosodisulfonate or salcomine, to obtain the compound of the present invention of

the general formula (Ia)



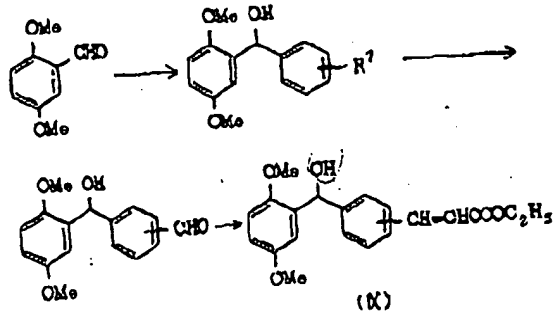
(Ia)

(wherein $R^1$, $R^2$, and $R^3$ are as defined above).

The compound of the present invention may be also produced by the following method:

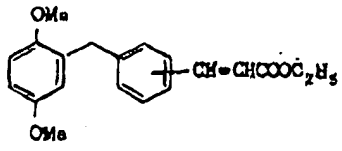
Process II

A compound of a general formula (IX) can be obtained from 2,5-dimethoxybenzaldehyde through the following route as described above.



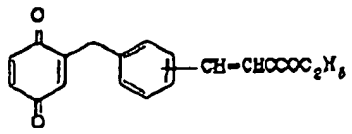
(IX)

The compound (IX) is converted into a chloride using thionylchloride, etc. and, then, is subjected to dechlorination, for example reduction with zinc-glacial acetic acid to obtain a compound of a formula (X).



(X)

The compound represented by a formula (Ib) of the present invention can be obtained by oxidation of the compound (X) with ammonium nitrate cesium (hereinafter abbreviated to CAN).



(Ib)

The compound (Ib) may be converted into various compounds of the present invention through hydrolysis, reduction, amidation, etc., as appropriate, under conventionally employed condition.

[EFFECT OF THE INVENTION]